SEQ ID NO.	Percent Identity to 17352471
146	79.3%
4	75.7%
6	75.9%
8	78.8%
10	99.3%

Sequence alignments and percent identity calculations were performed using the Megalign program of the LASERGENE bioinformatics computing suite (DNASTAR Inc., Madison, WI). Multiple alignment of the sequences was performed using the Clustal method of alignment (Higgins and Sharp (1989) *CABIOS.* *5*:151-153) with the default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=10). Default parameters for pairwise alignments using the Clustal method were KTUPLE 1, GAP PENALTY=3, WINDOW=5 and DIAGONALS SAVED=5. For large protein sequences the Clustal W program (Thompson et al. (1994) Nuc Acids Res 22:4673-4680) was used. Default parameters were used (GAP PENALTY=10, GAP LENGTH PENALTY=0.2, DELAY DIVERGENT SEQ (%)=30, DNA TRANSITION WEIGHT=0.50, PROTEIN WEIGHT MATRIX: Gonnet Series, DNA WEIGHT MATRIX:IUB). Pairwise alignment also used default parameters (GAP PENALTY=10, GAP LENGTH PENALTY=0.10, PROTEIN WEIGHT MATRIX: Gonnet 250, DNA WEIGHT MATRIX:IUB). Sequence alignments and BLAST scores and probabilities indicate that the nucleic acid fragments comprising the instant cDNA clones encode a substantial portion of a ryanodine receptor protein.

Figure 1 shows an alignment using Clustal W of the tobacco budworm, green peach aphid, cotton melon aphid, and corn plant hopper (SEQ ID NOs:2, 128, 130, 144, 146, 4, 6, and 8) to a fruitfly (*Drosophila melanogaster*) sequence generated by the methods disclosed herein (SEQ ID NO:10), the *Drosophila* art sequence ((NCBI General Identifier No. gi 17352471, SEQ ID NO:56), an unannotated sequence from the mosquito (*Anopheles gambiae*) genome sequencing project encoding a partial ryanodine receptor (gi 21301556, SEQ ID NO:57), the ryanodine receptor from nematodes (*Caenorhabditis elegans*, gi 1871447, SEQ ID NO:58), sea urchin (*Hemicentrotus pulcherrimus*, gi 18656155, SEQ ID NO:59), mouse (*Mus musculus*, gi 13569850, SEQ ID NO:60), rabbit (*Oryctolagus cuniculus*, gi 1245376, SEQ ID NO:61), and human (*Homo sapiens*, gi 4506757, SEQ ID NO:62). Insect specific sequences can be identified by finding homologous subsequences that are conserved between all of the insect sequences but not amoung the non-insect sequences. A number of these sequences can be found in SEQ ID NOs:63-119.

those that offer a variety of choices of sequences that when fused in-frame with the cDNA insert provide a polypeptide extension that can aid purification like a His tag, MBP or GST extension at either the N- or C-termini of the recombinant protein.

The cDNA inserts can also be expressed in yeast cells. The ryanodine receptor polypeptides can be evaluated by expression of the encoded polypeptides in a yeast (*Saccharomyces cerivisae*) strain YPH (Stratagene) and assaying the membrane components for ryanodine-sensitive calcium transport. Plasmid DNA (200 ng) from any of the cDNA clones can be used as a template for PCR using primers set forth in the present invention. Amplification is performed using the GC melt kit (Clontech) with a 1 M final concentration of GC melt reagent. Amplification took place in a Perkin Elmer 9700 thermocycler for 30 cycles as follows: 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 2 minutes. The amplified insert is then incubated with a modified pRS315 plasmid (NCBI General Identifier No. 984798; Sikorski, R. S. and Hieter, P. (1989) *Genetics 122*:19-27) that had been digested with appropriate restriction enzymes. Plasmid pRS315 had been previously modified by the insertion of a bidirectional gal1/10 promoter between the Xho I and Hind III sites. The plasmid is then transformed into the YPH yeast strain using standard procedures where the insert recombines through gap repair to form the desired transformed yeast strain (Hua, S. B. et al. (1997) *Plasmid 38*:91-96.).

Yeast cells are prepared according to a modification of the methods of Pompon et al. (Pompon, D. et al. (1996) *Meth. Enz. 272*:51-64). Briefly, a yeast colony is grown overnight (to saturation) in SG (-Leucine) medium at 30°C with good aeration. A 1:50 dilution of this culture is made into 500 mL of YPGE medium with adenine supplementation and allowed to grow at 30°C with good aeration to an OD_{600} of 1.6 (24-30 h). Fifty mL of 20% galactose is added, and the culture is allowed to grow overnight at 30°C. The cells are recovered by centrifugation at 5,500 rpm for five minutes in a Sorvall GS-3 rotor. The cell pellet is resuspended in 500 mL of 0.1 M potassium phosphate buffer (pH 7.0) and then allowed to grow at 30°C for another 24 hours.

The cells are recovered by centrifugation as described above and the presence of ryanodine receptor activity is determined using any of the assays outlined in Examples 9, 10, or 11.

EXAMPLE 7

Expression of *Heliothis virescens* Ryr cDNA in Insect Cells

The *Heliothis virescens,* and other, Ryr cDNAs appears to be quite toxic to Escherichia coli when placed into a vector under the control of an insect expression vector (e.g. pIB/V5-His, Invitrogen Life Technologies™, Carlsbad CA). Under these conditions, the cells grow very slowly and frameshift mutations are spontaneously

introduced. Two cloning strategies that completely eliminate any expression of the Ryr gene in E. coli would circumvent the growth problems.

In the first strategy, the Ryr gene is cloned into a modified pXINSECT-DEST39 vector (Invitrogen), placing the Ryr gene under the control of an insect actin promoter. In order to completely eliminate leaky expression of the Ryr gene during growth in E. coli, a "stop" fragment was placed just downstream of the His tag in the vector, but upstream of the Ryr gene. This stop fragment contains the strong transcriptional terminator T1T2 from the E. coli rrnB gene (Orosz et al (1991) *Eur J Biochem 201*:653-659). Also present within this stop fragment is a stop codon in frame with the initiator ATG of the vector. Flanking the stop fragment are Lox sites in direct orientation, such that in the presence of the Cre enzyme, the entire stop fragment will be excised from the vector (Odell and Russell (1994) Homologous Recomb Gene Silencing Plants; ed, J. Paszkowski; pp 219-270), leaving behind a "footprint" of a single Lox site. The stop vector was designed such that following Cre-mediated excision of the stop fragment, the Ryr gene is in frame with the initiator ATG and there are no stop codons present between the ATG and the Ryr gene. The combination of the ATG, His tag, and *attB1* from the vector and the residual Lox footprint and associated linker sequences, adds 53 foreign amino acids to the mature Ryr gene product. Construction of the pHE7 vector is outlined in Figure 7.

The Invitrogen Gateway Entry™ vector pEntr2B (Invitrogen Life Technologies™, Carlsbad, CA) was modified to add a PvuI site in the polylinker. pEntr2B was digested with EcoRI and KpnI. Two oligonucleotides, SEQ ID NO:131 (5'-gatccgcggccgcgatcggtacc-3') and SEQ ID: 132 (5'-cgatcgcggccgcg -3') were annealed and ligated into the digested vector to produce pEntr2B/PvuI. The HV Ryr gene from pXLHv7 was digested with PacI and PmeI. pENTR2B/PvuI was partially digested with PvuI (there is a second site in pEntr2B/PvuI) and completely digested with EcoRV and the correct fragment was gel-purified. The PacI sticky end of the Ryr fragment is compatible with the PvuI sticky end and the PmeI and EcoRV ends are both blunt. The 15.4 kb Ryr gene fragment from pXLHv7 was then ligated into the pENTR2B/PvuI to create pENTR2B/PvuI/HV7.

The stop fragment was created by annealing 6 oligonucleotides, SEQ ID NO: 133 - SEQ ID NO:138 and then PCR amplified using primers SEQ ID NO:139 (5'-ccttttttttcagcgcta-3') and SEQ ID NO:140 (5'-gagagagagtttaaataa-3'). The resulting product was digested with AfeI and DraI to produce blunt ends, and ligated into the unique AfeI site of pXINSECT-DEST39 to produce pDEST39/LOX.

As an alternative to using pDEST39/LOX, the stop fragment from pDEST39/LOX was excised with XbaI and HindIII and cloned into the pIB/V5-His-